REMARKS

This is in response to the Office Action mailed July 24, 2003, and further to the Notice of Appeal filed January 26, 2004 and the Request for Continued Examination accompanying this submission. The Advisory Action mailed March 12, 2004 stated that the response filed October 9, 2003 had not been entered.

In this response, claims 1 and 12 have been amended, and new claim 21 has been added. Thus, upon entry of this response, claims 1, 4-10, and 12-21 will be pending.

Claim 1 has been amended to recite a method for preventing T cell mediated tissue destruction associated with type I diabetes, wherein the tissue destruction results from a cell-mediated immune reaction to self. This is supported by the specification at, e.g., page 3, last paragraph. Claim 12 has been amended to independent form. New claim recites a particular embodiment of the methods of claim 1, supported at, e.g., pages 3-4, bridging paragraph, and

No new matter has been added by way of this response. Each of the Examiner's rejections is addressed below.

Anticipation

To meet the burden of anticipation under 35 U.S.C. §102, a single prior art reference must disclose each and every element of the rejected claim(s). See, M.P.E.P. § 2131. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Every element of the claimed invention must be literally present, arranged as in the claim. *Perkin Elmer Corp.* 732 F.2d 888, 894, 221 USPQ 669, 673.

1. Noelle '693 Patent

Claims 1 and 4-10 stand rejected as allegedly anticipated under 35 U.S.C. §102(e) by U.S. Patent No. 5,683,693 to Noelle et al. ("Noelle '693 patent"). The Examiner argues that the Noelle '693 patent teaches the substance of the invention, and that the claimed functional limitations would be inherent properties of the referenced methods to treat diabetes, referring to the following section of the Noelle '693 patent (col. 11, ll. 32-39):

> Accordingly, the invention encompasses a method for treating diabetes by pancreatic islet cell transplantation. The method comprises administering to a subject in need of treatment: 1) allogeneic or xenogeneic cells which express donor antigens, 2) an antagonist of a molecule expressed on recipient T cells which mediates contact-dependent helper effector function, such as a gp39 antagonist (*e.g.*, anti-gp39 antibody) and 3) donor pancreatic islet cells.

This rejection is respectfully traversed. The method described in the Noelle '693 patent would not, inherently or otherwise, lead to inhibition or prevention of T cell mediated tissue destruction from a cell-mediated immune reaction to self (*i.e.*, an autoantigen). By contrast, since the method in the Noelle '693 patent is based on administration of allogenic donor cells in conjunction with a gp39-antagonist, the method results in inhibition of an immune response to foreign antigens (*i.e.*, donor or alloantigen). The Noelle describes its method as follows (col. 2, ll. 58-60; emphasis added):

> Induction of T-cell tolerance to alloantigens as described herein can be used as a preparative regimen for tissue or organ transplantation.

There is no description or suggestion in the Noelle '693 patent to modify the method described therein to exclude the administration of donor antigen or donor antigen-expressing cells for the purpose of achieving an inhibition of a response to self, or for some other purpose. Absent such a teaching or suggestion, the Noelle '693 patent cannot anticipate the present claims, which call for a method for preventing T cell mediated tissue destruction associated with type I diabetes by administering to a subject a therapeutically or prophylactically amount of a gp39 antagonist.

2. Lederman patent

Claims 1 and 4-10 also stand rejected as anticipated under 35 U.S.C. §102(e) by U.S. Patent No. 5,993,816 to Lederman et al. ("Lederman patent"). Specifically, the Examiner contends that the Lederman patent teaches the use of 5c8-antigen/CD40L-specific antibodies to treat autoimmune diseases including diabetes, and that the claimed functional limitations would be inherent properties of the referenced methods to treat type I diabetes with 5c8-antigen-specific antibodies. The Examiner refers to the following section of the Lederman patent:

> In another embodiment of this invention, inhibiting the immune response of an animal is valuable as a method of inhibiting the autoimmune response in an animal suffering from autoimmune disease. Examples of autoimmune diseases include, but are not limited to, **rheumatoid arthritis, Myasthenia gravis, systemic lupus erythematosus, Graves' disease, idiopathic thrombocytopenia purpura, hemolytic anemia, diabetes mellitus** and drug-induced autoimmune diseases, e.g., drug-induced lupus.

This rejection is respectfully traversed on the ground that the skilled artisan at the time of the invention would not have found the Lederman patent's claim for preventing tissue-mediated destruction in an autoimmune disease, such as those listed, as credible. Since an anticipatory reference must fully enable the invention (*Elan Pharmaceuticals, Inc. v. Mayo Foundation for Medical Education and Research*; 345 F.3d 1051, 1052, 68 USPQ2d 1373 (Fed. Cir. 2003). See also *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 USPQ2d 1385, 1416 (Fed.Cir.2003)), and since an anticipating reference must describe the...subject matter with sufficient clarity and detail to establish that the subject matter existed and that its existence was recognized by persons of ordinary skill in the field of the invention (*ATD corp. v. Lydall, Inc.*, 169 F.2d 534, 48 USPQ2d e1321 (Fed. Cir. 1998)), a hypothetical statement, absent other instruction, cannot be enabled. Thus, the Lederman patent cannot anticipate any of the claims since, as discussed below, there is no teaching that would enable one to leap to the conclusion that the 5c8 antibody could prevent T cell mediated tissue destruction in primarily T cell mediated autoimmune diseases.

In fact, subsequent evaluation of Lederman's humanized 5c8 antibody, when evaluated in a clinical setting, has been described in an article in the American Journal of Transplantation

(Vol. 2, Issue 10, Page 898, November 2002-**Exhibit A**) as "*the most dramatic failure in recent past.*" Clinical studies of were halted due to thromboembolic events and failure of efficacy (5 out of 7 patients experienced graft rejection). The article continues by describing evaluation of the presently claimed antibody (IDEC-131/E6040), which targets a different epitope than Lederman's hu5C8 antibody, in autoimmune diseases. IDEC-131, in contrast to hu5c8, exhibits no severe adverse events but is safe and well-tolerated (see documents at **Exhibit B**).

Since the present claims are amended to recite that the antibody is produced by hybridoma HB11713 (i.e., IDEC-131-see **Exhibit C**), the Lederman cannot anticipate. Accordingly, withdrawal of this rejection is respectfully requested.

In addition, the Lederman patent does not anticipate for the following further reasons.

The Lederman patent is primarily concerned with inhibiting T cell activation of B cells (col. 10, l. 60 to col. 11, l. 5), and also with detecting T cell blood tumors by detecting binding of the 5c8 antibody to the tumor (col. 2, ll. 42-53), thereby inhibiting proliferation of the tumor (col. 13, l. 38-37). There is absolutely **no teaching** of inhibiting T-cell mediated tissue destruction, much less inhibiting T-cell mediated β-cell destruction. From the meager teachings in Lederman, which are exclusively directed to "helper" T cell function, i.e., preventing a B-cell mediated humoral immune response, it cannot even be deduced that the antibody could effect T cell mediated tissue destruction that came prior to the humoral response, much less be taken as an explicit teaching of Lederman. This is supported by Lederman's teaching at col. 27, ll. 49-52, which states that:

> "The heterogeneous distribution of 5c8 expression on activated CD4+ cells suggests that functional subsets of CD4+ T cells might be distinguished by their level of 5c8 expression. For example, it will be of interest to determine the functional potential of 5c8- and 5c8+ CD4+ T cells with respect to helper or cytotoxic activity."

By this statement, Lederman conveys that T cells having cytotoxic activity are likely **distinct** from those effecting T cell helper activity with respect to 5c8 expression. Thus, this in

effect, teaches away from a suggestion that administration of an anti-5c8 antibody would inhibit T-cell mediated tissue destruction.

Moreover, the named autoimmune diseases in Lederman, rheumatoid arthritis, Myasthenia gravis, systemic lupus erythematosus, Graves' disease, idiopathic thrombocytopenia purpura, hemolytic anemia, and diabetes mellitus, were all known to have a **humoral** (autoantibody-mediated) pathology, consistent with Lederman's strategy of inhibiting T cell-mediated B cell activation, and hence, autoantibody production. This is evidenced by the following abstracts (attached at **Exhibit D**) in view of each named disease: **Reiter** et al., *Arthritis Rheum* 1991; 24: 525-36 ("Recent experimental an clinical data point to the T helper lymphocyte subset as playing a central role in the pathogenesis of rheumatoid arthritis (RA)"; **Reim** et al., J Neuroimmunol. 1992; 41: 61-70 ("The pathogenesis of myasthenia gravis (MG) involves a T cell-dependent antibody-mediated autoimmune response directed against acetylcholine receptors (AChR)"); **Singh**, Med Hypotheses. 1992; 39: 356-9 ("Excessive production of pathogenic autoantibodies of one of the hallmarks of systemic lupus erythematosus (SLE)"); **Semple** et al., Autoimmunity 1992; 13: 311-19 ("Chronic autoimmune thrombocytopenic pupura is a common autoimmune-mediated bleeding disease in which autoantibodies are directed against platelets..."); **Fisfalen** et al., J. Clin. Endocrinol Metab. 1990; 71: 170-78 ("Our antigen specific [T cell clone] could...stimulate specific B-cells to produce thyroid antibodies in vitro. Self MHC-II-reactive [T cell clones] could also induce specific antibodies by B-cells."); and **Landin-Olssen** et al., Diabetologia. 1992. 35(11):1068-73 ("Among [IDDM] patients, the frequency of islet cell antibodies was 84%... insulin autoantibodies 43%...; 40%...were positive for both and 88%....were positive for one or both. Among control children, 3% ...had islet cell antibodies, 1...insulin autoantibodies, and 4%...had either autoantibody marker.)"

These teachings support the disclosure of Lederman's patent, and hence, Lederman's invention, that the 5c8 antibody would be useful to prevent T cell mediated B cell antibody production. However, based on Lederman's disclosure, there is not even a hint that T cells mediate tissue destruction in any of these diseases, much less IDDM. Nor is there any teaching that, particularly for IDDM, prevention of T cell mediated responses are crucial for treatment of the

disease pathology. As indicated by the enclosed literature, further discussed immediately below, the humoral antibody response is not the mechanism by which b-cell destruction occurs. To the contrary, many of the antibodies seen in IDDM likely arise as a secondary event, following tissue destruction and release of antigens which are normally intracellular, and hence, foreign. Inhibiting production of these antibodies would have no benefit in IDDM, since by then, the damage is done.

It has previously been pointed out that the Lederman patent relates to the use of a 5c8/CD40L antibody to inhibit B-cell activation and humoral immune responses, and that, by contrast, the destruction of β-cells in type I diabetes is triggered and mediated primarily through a T-cell mediated cellular autoimmune response (see Amendment dated May 9, 2003, p. 8, 1st paragraph), citing and enclosing the abstracts of Casares et al. and Campbell et al. (Full copies of these references are provided in the accompanying Information Disclosure Statement, filed concurrently herewith).

Thus, the art-recognized mechanism for induction of the autoimmune mechanism responsible for the β-cell destruction in type I diabetes has long been that β-cells antigens were released, possible because of some injury; and that this would trigger an autoreactive T cell response against the β-cells (see, e.g., Casares et al., p. 362, Figure 4). However, what was also established at the time was that the humoral immune component emerged after this initial, T-cell mediated tissue destruction had begun Knip et al., *Ann Med* 1997; 29: 447-51 (**Exhibit E**) ("[selective destruction of pancreatic *β*-cells]...is assumed to be T cell mediated, but the emergence of disease-associated autoantibodies in to the peripheral circulation is usually the first noticeable sign of beta-cell autoimmunity in IDDM"). In other words, the humoral immune component was also what was used as a first clinical sign that the disease pathology was, in fact, in progress. Accordingly, even though the Lederman patent hypothesized that an antibody against the 5c8 antigen/gp39 could be used to "inhibit the autoimmune response in an animal suffering from autoimmune disease," the skilled artisan would not have found this credible since:

- The "animal" in the referenced section of the Lederman patent *already suffers* from an autoimmune disease such as diabetes;

- In an animal already suffering from diabetes, pancreatic β-cell destruction is almost complete; and

- If β-cell destruction is almost complete, administering a compound postulated to be capable of inhibiting a humoral immune response, *i.e.*, the Lederman patent's 5c8 antibody would have *no effect on the cell-mediated tissue destruction.*

These assertions are supported by literature references (Knip, *Acta Paediatr* 1998, Supp 425:54-62) which demonstrates that, by the time diabetes is diagnosed, end-stage insulitis (T cell infiltrates in islets) is occurring, thus precluding the possibility of preventing insulitis. According to Knip, 80-90% of pancreatic β-cells are destroyed by end-stage insulitis. Another article by Hanninen et al., demonstrates the presence of T cell cells, macrophages and other mononuclear cells involved in tissue destruction in cross-sections from a pancreas at the onset of IDDM (*J. Clin. Invest.* 1992; 90: 1901-10).

Further, as amended, claim 1 does not recite "inhibiting" a T-cell mediated tissue destruction associated with type I diabetes, and claim 1 now recites that the anti-gp39 antibodies are useful for *preventing* tissue-mediated destruction. Since these antibodies are distinct from those described by Lederman (and bind different epitopes-see **Exhibit A**), this is another reason why Lederman cannot anticipate the present claims.

In summary, while it was known at the time the Lederman patent was filed that the pathology of IDDM had a T cell-mediated component (see Abstract by Bach, Curr Opin Immunol. 1991; 3: 902-5), Lederman makes no mention of this in the '816 specification of addressing such pathology using the 5c8 antibody. Moreover, as indicated above, preventing the T cell-mediated antibody response in an IDDM patient would be pointless, since pancreatic tissue destruction has already occurred by the time the humoral response manifests.

As outlined above, neither the Noelle '693 patent nor the Lederman patent anticipates the claims as amended herewith. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Obviousness

The Examiner maintains claims 1, 4-10, and 12-20 as allegedly obvious over the Noelle '693 patent and/or the Lederman patent in view of U.S. Patent No. 5,747,037 to Noelle et al. ("Noelle '037 patent). Specifically, the Examiner contends that "given the antagonistic properties of 24-31 and 89-76 CD40L specific antibodies taught by Noelle et al. ('037), the ordinary artisan would have been motivated to substitute these CD40L antagonists into the methods of treating autoimmune diseases such as diabetes, as taught by Noelle et al. ('693) and Lederman" (office action, p. 4).

This rejection is respectfully traversed, since the effective priority date of the 24-31 and 89-76 antibodies described in the Noelle '037 patent is June 7, 1995, which is the same date as the priority date of the present claims. Accordingly, the Noelle '037 patent teachings relied on the Examiner for this rejection are not available as prior art. Neither the Noelle '693 patent nor the Lederman patent makes up for this missing teaching, as they do not describe or suggest the 24-31 or 89-76 antibodies.

Further, as described above, the method taught in the Noelle '693 patent would not have inhibited or prevented tissue destruction from a cell-mediated reaction to **self antigens,** and does not suggest modification of this method to obtain such a method. Similarly, the Lederman patent fails to provide this missing teaching since Lederman does not teach T cell mediated tissue destruction, but only T cell mediated B cell activation (i.e., the humoral component). To the contrary, the instant application expressly teaches that IDDM is a T cell mediated disorder, and that the **protective** effect conferred by transplanting donor spleen cells from animals immunized with PLP-peptides (in the EAE animal model of T cell mediated autoimmunity) was due to the suppression of the activated T cells (page 6, col. 2, top).

Lastly, one of ordinary skill in the art would not have been motivated by the teachings of Noelle '693 or Lederman since neither disclose preventing T cell destruction, and thus, in combination would not permit one of ordinary skill in the art to arrive at the presently claimed invention.

For all of the above reasons, it is respectfully requested that this rejection be withdrawn.

Conclusion

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

By _____

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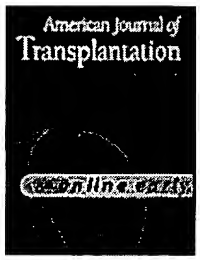

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What's New - What's Hot

What's in the Pipeline? New Immunosuppressive Drugs in Transplantation

Flavio Vincenti

In the pipeline, there are a number of novel immunosuppressive drugs in preclinical development or in early clinical trials. The major target of new agents are cell-surface molecules important in immune cell interactions (especially the costimulatory pathway), signaling pathways that activate T cells, T-cell proliferation and trafficking and recruitment of immune cells responsible for rejection. The most promising biologic agents include a humanized anti-CD11a (anti-LFA1), humanized anti-B7.1/B7.2, a second-generation CTLA4Ig (LEA29Y) and a humanized antibody to anti-CD45 RB. Inhibitors of T-cell activation and signaling are still in preclinical development. The most interesting inhibitors of T-cell proliferation include inhibitors of the Janus protein tyrosine kinase, JAK3, and FK778, a leflunomide analog. Chemokines play an important role in rejection by virtue of their critical role as regulator of trafficking and activation of lymphocytes. Early trials of FTY720, a synthetic small molecule with functional homology to sphingosine-1 phosphate leading to lymphocyte sequestration, appear very promising; however, enthusiasm for this drug is mitigated by its potential cardiac side-effects. Antagonists to several chemokine receptors, including CCR1, CXCR3 and CCR5, have been shown to be effective in experimental transplantation and are likely to be considered for clinical development.

Introduction Go to: Choose Go

The past decade has witnessed important advances in transplantation in terms of therapeutic modalities and improved short-term and long-term outcomes. Impressive numbers of experimental drugs were brought to the clinic, had successful phase III trials and were approved for clinical use. Cyclosporine, the drug that ushered in the renaissance era of transplant therapeutics, was replaced by a microemulsion formulation with more predictable pharmacokinetics (1). A recent analysis showed that patients treated with the microemulsion cyclosporine formulation had better long-term graft outcome than patients treated with Sandimmune (2). A second calcineurin inhibitor, tacrolimus, was successfully introduced for both liver and renal transplantation (3,4). Two antiproliferative drugs, mycophenolate mofetil (MMF) and sirolimus, with different mechanisms of action were introduced in 1995 and 1999, respectively, and have had a dramatic effect on the reduction of the incidence of acute rejection in renal transplantation (5,6). There were also important advances in biologic induction therapies. A new generation of monoclonal antibodies (mAbs) targeting the α chain of

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the interleukin-2 receptor was shown in phase III trials to result in selective and effective immunosuppression with a safety profile unmatched by any other immunosuppression agent (7,8). Finally, the biologic depleting agent antithymocyte globulin (Thymoglobulin) that has been available in Europe for over a decade was finally approved in the United States for use in the treatment of steroid-resistant acute rejection (9). It has replaced OKT3 as the depleting antilymphocyte agent of choice for induction therapy, especially in high immunologic risk patients.

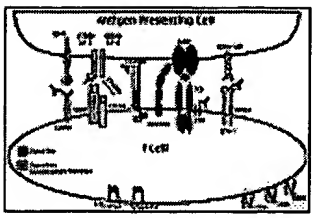

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Figure 1: Cell-surface targets of biologic agents.

The drugs and biologic agents which will be introduced in the next decade will be propelled by the evolution of our understanding of the pathways that lead to rejection, tolerance, tissue repair, as well as by the ever-expanding genomic discoveries. Ironically, it will be several years before the next new drug will be approved for transplantation, as currently there are no phase III trials under way with new drugs or biologic agents. The success of the existing immunosuppression regimens may hinder future drug development because of the vanishing endpoint of acute rejection, and the challenge of demonstrating improvement in long-term outcome (10). Meanwhile, several unmet clinical needs persist. These include patients at high risk of rejection and graft loss, such as African Americans, patients with high panel-reactive antibodies, as well as patients who develop delayed graft function. Thus the thrust of future drug development is likely to result in immunosuppression therapy with a unique or novel mechanism of action that, while not necessarily resulting in superior short-term efficacy, will lack current drug toxicities and lead to better preservation of renal function. The promise of immunologic tolerance hovers on the horizon unfulfilled but not beyond reach, and convincing nonhuman primate models and the use of a combination of experimental drugs or biologic agents in clinical trials will be required for further progress. The recently established NIH-sponsored Immune Tolerance Network will be an important catalyst in promoting clinical experimentation with tolerance-inducing regimens.

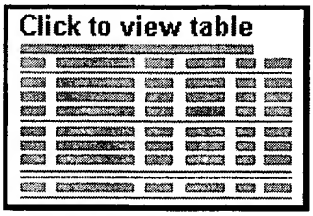

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Table 1: Major targets of new agents in development

New Targets for Immunosuppression Go to: Choose Go

Table 1 shows the major targets of new agents in immunosuppression in clinical and preclinical studies.

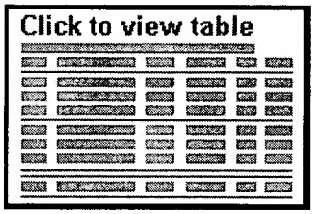

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Table 2: Efalizumab dose and concomitant immunosuppression

Interference with cell-surface molecules important in immune cell interactions

Targeting cell-surface molecules with biologic agents has several advantages over maintenance oral drug therapy. Cell-surface molecules can be readily blocked with monoclonal antibodies or receptor-fusion proteins, and are easily saturated and modulated. The new humanized biologic agents have the added advantage of a long half-life, requiring infrequent administration. In addition, there is a paradigm shift in biologic drug development from short-term induction therapy to chronic administration as a replacement for maintenance oral immunosuppression. The potential advantage of chronic biologic therapy is regimen simplification (monthly or bi-monthly administrations), no requirement for therapeutic drug monitoring, and assured compliance.Figure 1 shows some of the current biologic agents in early clinical trials or being considered for clinical development. Other biologic agents that are being used in single-center trials but are not yet part of a formal drug development program by their sponsor include campath 1H, a humanized anti-CD52 antibody that results in prolonged lymphocytes depletion; rituximab, an anti-CD20 monoclonal antibody that targets B cells; and several humanized mutagenized nonmitogenic anti-CD3 mAbs(11-16). Kirk et al. and Knechtle et al. are conducting single-center trials with campath 1H in combination with sirolimus in an attempt to induce long-term tolerance or at the very least drug minimization. Rituximab is being used off-label in patients with elevated levels of panel-reactive antibodies as well as in patients undergoing acute humoral rejection (13). The new generation of humanized anti-CD3 mAbs are engineered to loose their toxicities through amino acid(s) substitution in the Fc domain in order to reduce binding to Fc receptors. These antibodies include HuM291, Campath 3 and hOKT3 γ1 (the humanized-mutagenized version of murine OKT3)(14-16). hOKT3 γ1 is licensed to Centecor and is being considered for use in new trials in renal transplantation.

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The most dramatic failure in the recent past is Biogen's anti-CD154 (hu5C8) (17). Despite impressive experimental evidence, the phase I trial with the humanized hu5C8 was halted following thromboembolic events, as well as failure of immunosuppression

efficacy (5/7 patients had rejection episodes). IDEC Pharmaceutical has started clinical trials with another humanized anti-CD154, IDEC131 (targeting a different epitope than hu5C8) in patients with autoimmune diseases, but may extend these studies to organ transplantation pending results in nonhuman primates. It is clear that there is continuing interest in exploring disruption of the CD40 pathway in clinical trials.

A humanized antibody to CD45 (anti-CD45RB) may soon put the spotlight back on CD45 as an important drug target. CD45 was first described in 1978 as a family of glycoproteins expressed on the surface of nucleated hematopoietic cells (18). CD45 is a transmembrane protein tyrosine phosphatase involved in the coupling of signals from the T-cell receptor to the proximal signaling apparatus. Different CD45 isoforms generated by the alternative slicing of exons A, B and C are expressed by T cells with distinct functions. Monoclonal antibodies to the RB isoforms of CD45 have been shown to induce long-term survival and tolerance in various experimental models of solid organs and islet cell transplant(19-21). The mechanism of action of anti-CD45RB mAbs is unclear. However, it may modulate the expression of RB isoforms with different molecular weights (m.w.) (21). T cells with high m.w. CD45RB ($CD45RB^{bright}$ cells) secrete IL-2, while low m.w. CD45RB ($CD45RB^{dim}$ cells) secrete Il-4. Basadonna et al. showed that lymphocytes obtained from animals treated with anti-CD45RB showed decreased $CD45RB^{bright}$ cells and had up-regulation of $CD45RB^{dim}$ cells (19). In addition,*in vitro* effects of anti-CD45RB mAbs include down-regulation of theL-selectin, up-regulation of CTLA-4, and suppression of TH1 cytokine production. In a seminal study, Lazarovits et al. showed that an anti-CD45RB mAb in two doses resulted in long-term graft survival in murine renal allografts (20). Non-human primate models are currently underway in preparation for a phase I clinical trial in renal transplant recipients by Abgenix.

Efalizumab is a humanized IgG1 monoclonal antibody targeting the CD11a chain of LFA1. Efalizumab binds to LFA1, preventing LFA1-ICAM interaction. Direct blockade of ICAM-1 with a mAb failed to show any benefit in a randomized renal transplant trial, possibly due to redundancy in the ICAMs (22) Anti-CD11a has been shown to block T-cell adhesion, trafficking and activation (23). Pre-transplant therapy with anti-CD11a prolongs survival of murine skin and heart allografts, and monkey-heart allografts (24). Efalizumab has been successfully used in phase III trials in patients with psoriasis. In a phase I/II open label, dose ranging, multidose, multicenter trial, Efalizumab was administered subcutaneously, weekly for 12 weeks following renal transplantation (25).Table 2 shows patient enrollment as well as the maintenance immunosuppression. At 3 months, 3/38 patients (7.8%) had a reversible rejection episode and at 6 months there was one additional rejection for a cumulative rejection rate of 10.4%. Pharmacokinetic and pharmacodynamic studies showed that the lower doses of Efalizumab (0.5 mg/kg) produced saturation and 80% down-modulation of CD11a by 24-48 h following therapy. In a subset of 10 patients who received the high-dose Efalizumab (2 mg/kg) with full-dose cyclosporine and MMF, 3/10 patients developed post-transplant lymphoproliferative disease. Thus Efalizumab appears to be an effective immunosuppressive agent, but it is best used in a lower-dose regimen with less intense maintenance immunosuppression.

The costimulatory pathway also referred to as signal two (signal one being the antigen-driven pathway via the T-cell receptor) is critical in triggering T-cell activation, proliferation and effector function(26-29). While many coactivation or costimulatory pathways have been described (CD154-CD40, LFA_1-ICAM-1, ICOS-B7RP-1) the CD28-B7 interaction remains the most thoroughly characterized and possibly represents the best target of immunosuppression therapy. Despite the failure of anti-CD154 in a single clinical trial, blocking CD28 interaction with B7 (either with CTLA4Ig or with anti-B7 mAbs) will continue to be an important focus of clinical studies.

h1F1 and h3D1 are humanized anti-B7.1 (CD80) and B7.2 (CD86). The DNA encoding the complementarity determining regions from the murine antibodies were molecularly spliced on to the DNA for the human kappa light, and the DNA for the $\gamma 2$ heavy chain sequences mutagenized to minimize Fc binding.*In vitro* h1F1 and h3D1 were shown to block CD28-dependent T-cell proliferation and decrease mixed lymphocyte reactions. In nonhuman primate models, h1F1 and h3D1 were able to delay renal allograft rejection, and their effectiveness was not undermined by the use of calcineurin inhibitors or steroids (30). The monoclonal antibodies need to be used in tandem, since either B7.1

or B7.2 is sufficient to stimulate T cells via CD28. A single phase I study in renal transplant recipients was performed in patients receiving maintenance therapy consisting of cyclosporine, mycophenolate mofetil and steroids. Patients received a single pretransplant dose ranging from 0.15 mg/kg to 5 mg/kg. Though the results of the study are yet to be published, the preliminary results appear to show that these monoclonal antibodies were safe and effective. While Wyeth Pharmaceutical at the present time has decided not to proceed with further development of these antibodies, they may yet emerge in the future through licensing agreements.

LEA29Y is a second-generation CTLA4Ig (extracellular domain of CTLA4 and IgG1 Fc domain) with an increase in binding avidity to CD80 (2-fold) and CD86 (4-fold), and approximately 10-fold more effectiveness*in vitro* than CTLA4Ig on a per dose basis in inhibiting T-cell effector functions. A phase I/II trial is currently underway in primary renal transplants with an immunosuppression regimen based on preclinical studies performed by Drs Chris Larsen and Tom Pearson at Emory University (unpublished results). In the phase I study, 210 primary renal transplant patients are randomized to three treatment groups, group 1 and group 2 are treated with different regimens of LEA29Y, basiliximab (20 mg day 0 and day 4), mycophenolate mofetil 2 g and conventional steroid therapy. Patients randomized to group 3 serve as controls and are treated with a standard regimen consisting of basiliximab (20 mg at day 0 and day 4), cyclosporine, mycophenolate mofetil, and steroids. Patient enrollment in this trial should be completed by December 2002. This study may provide an important clue to the clinical efficacy achieved by blocking a single tract of the costimulatory pathway. It is possible, though, that effective clinical blockade of the costimulatory signal may require disruption of several targets within the pathway (29).

Inhibitors of T-cell activation and signaling

T-cell receptor (TCR)-coupled signaling in addition to costimulation delivered by CD28 activation results in activation of a number of signaling pathways that ultimately culminate in T-cell activation and cytokine production (31). Pre-clinical and*in vitro* inhibitors of signaling protein kinases for Lck, ZAP-70, PKC-θ, as well as MAPK cascade, are available. ZAP-70 inhibitors are particularly interesting in view of ZAP-70 s selective expression in T lymphocytes and natural killer cells. Inhibitors of the calcium-release-activated Calcium channel (CRAC) as well as specific inhibitors of NFAT (nuclear factor of activated T cells) rather than the phosphatase calcineurin may also be effective targets for inhibiting T-cell activation. Whether any of these agents will be developed clinically depends on a variety of factors, including imparting bioactivity and selectivity to these small molecules*in vivo*.

Inhibitors of T-cell proliferation

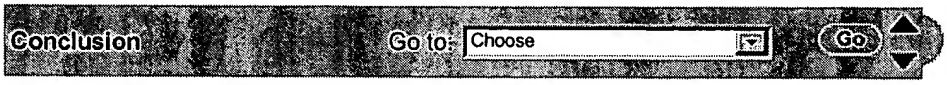
Effective T-cell activation requires T-cell proliferation. The new anti-interleukin-2 α chain receptor monoclonal antibodies cannot completely block T-cell proliferation as proliferative signals may occur through the intermediate affinity interleukin 2 receptor β γ or through pathways that involve cytokines other than IL2. The current approaches to blocking T-cell expansion are the disruption ofcytokine signaling or the inhibition of nucleotide incorporation required for cellular proliferation. Signaling through the γ chain requires activation of the Janus protein tyrosine kinase, JAK3, which also mediates signals from receptors for IL4, IL7, IL9 and IL15. Since JAK3 is required for the transduction of proliferative signals, inhibitors of JAK3 can be potentially powerful and useful drugs in transplantation (32). Individuals genetically lacking JAK3 have severe immunodeficiency disease (33). Whether JAK3 inhibitors turn out to be prohibitively immunosuppressive remains to be determined. Several JAK3 inhibitors, including one from Pfizer Pharmaceuticals, are development candidates for transplantation.

Novel antimetabolites include Lilly's Gemcitabine, a pyrimidine synthesis inhibitor currently being tested in a miniature swine model for renal transplantation, and FK778. FK778, a new oral immunosuppressive agent under development by Fujisawa Healthcare Inc., is an analog of the active metabolites of leflunomide (34). FK778 has a unique mechanism of action, binding to dihydro-orotate dehydrogenase and inhibiting de novo pyrimidine biosynthesis, thereby blocking T- and B-cell proliferation and strongly suppressing IgM and IgG antibody production. In addition, FK778 appears to have antiviral effects, including the polyoma virus. FK778 is currently in a phase II trial in Europe. A new, rationally designed inhibitor of inosine monophosphate dehydrogenase, VX-497, with a mechanism of action similar to mycophenolate mofetil,

has been developed by Vertex and used in clinical studies in patients with psoriasis and hepatitis C. Despite encouraging preclinical studies in renal transplantation in dogs, however, its clinical development in transplantation remains in doubt.

FTY720 is a synthetic structural analog of myriocin, a metabolite of an ascomycete. FTY720 shares structural and functional homology with sphingosine-1-phosphate (S1P), a natural ligand to several G-protein-coupled receptors. FTY720 displays a novel mechanism of action characterized by sequestering of lymphocytes into secondary lymphoid organs without affecting their functions or properties (35). FTY720-monophosphate (FTY720-P), the active form of the drug, acts as an agonist and signals the S1P receptor family, $S1P_4$ and $S1P_5$ on lymphocytes, thereby increasing the intrinsic mobility of the cells and their responsiveness to chemokines (36,37). Thus the FTY720P-triggered lymphocytes are sequestered to sites of high constitutive homing chemokine expression, namely lymph nodes and Peyers patches. The sequestration to the lymphoid system reduces migration of effector cells to inflammatory tissues and graft sites. In a recently published study, 20 stable renal transplant recipients on a cyclosporine-based regimen were treated with single oral doses of FTY720 ranging from 0.25 to 3.5 mg (38). FTY720 was well tolerated with no serious adverse events, except for transient asymptomatic bradycardia in 10/24 doses. The elimination half-life ranged from 89 to 157 h independent of dose. FTY720 pharmacodynamics were characterized by reversible transient lymphopenia within 6 h, the nadir being 42% of baseline. The lymphocyte count returned to baseline within 72 h in all dosing cohorts except the highest. The interim results of two FTY720 trials were reported at the 2002 ATC meeting in Washington DC (39,40). The first study assessed the efficacy of four different maintenance doses of FTY720 (0.25 mg, 0.5 mg, 1 mg, 2.5 mg) in 155 patients concomitantly treated with cyclosporine and prednisone (39). The incidence of acute rejection ranged from 38% to 11%. The second trial was performed in patients at risk for delayed graft function and the immunosuppression regimen consisted of FTY720 2 mg, RAD 2 mg and prednisone (40). Fifty-six patients were enrolled in this study, with a reported rejection rate of 19% (mean follow-up 176 days). Both trials have stopped enrollment following several episodes of severe bradycardia (probably mediated by activation of S1P receptors on atrial myocytes). New phase II and phase III trials are being considered for initiation in 2003. FTY720 appears to be an excellent candidate for rational drug design to eliminate its side-effects.

Chemokines play an important role in rejection by virtue of their critical role as regulators of trafficking and activation of lymphocytes (41,42). The receptors for chemokines are G-protein-coupled receptors expressed on a variety of leukocytes. Studies using targeted disruption of specific chemokine receptors, whether with receptor knock-out models, or receptor blockade with antagonists or monoclonal antibodies, have resulted in prolongation of allograft survival in experimental animals(42-46). These approaches appear to be more effective than neutralizing the cytokines with antibodies. Of approximately 18 chemokine receptors, at least three appear to play important roles in rejection in experimental models: CCR1, CXCR3, CCR5 (42,44,45). In experimental studies the chemokine receptor antagonists are more effective when used with low-dose calcineurin inhibitors than when administered as monotherapy. Development programs are currently in place for antagonists of CXCR3 and CCR1. While transplantation may not be the first indication in the clinical development of these agents, it is clear from experimental models that blockade of chemokine receptors could become an important addition to the therapeutic armamentarium in the prevention of rejection.



The next decade holds the promise of delivering newer, safer and more effective therapies to prevent acute and chronic rejection in organ transplantation. A pharmacologically promoted state of antigen-induced tolerance may even finally be achieved. In the interim, with no prospects for new drugs, transplant physicians should continue to experiment with immunosuppressive regimens, using currently approved drugs and biologic agents to minimize toxicities and improve long-term outcomes.

Acknowledgments

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IDEC 131: adverse reactions

Various toxicities

In patients with relapsing-remitting multiple sclerosis

Study Details

Purpose

This study investigated the tolerability of IDEC 131 [αCD154] in patients with relapsing-remitting multiple sclerosis.

Details

Design:	sequential
Control:	baseline comparison, drug dosage comparison
Phase:	Phase I
Concomitant Medication:	None

Subjects

Type	No.	Sex	Age
patients	12		not stated

Treatments

IDEC 131 (1 mg/kg)

Drug/Treatment	Dose	Route	Frequency	Duration
IDEC 131	1 mg/kg/dose	not stated	2/week	4 doses

IDEC 131 (5 mg/kg)

Drug/Treatment	Dose	Route	Frequency	Duration
IDEC 131	5 mg/kg/dose	not stated	2/week	4 doses

IDEC 131 (10 mg/kg)

Drug/Treatment	Dose	Route	Frequency	Duration
IDEC 131	10 mg/kg/dose	not stated	2/week	4 doses

IDEC 131 (15 mg/kg)

Drug/Treatment	Dose	Route	Frequency	Duration
IDEC 131	15 mg/kg/dose	not stated	2/week	4 doses

Results

Multiple doses of IDEC 131 at 1 mg/kg, 5 mg/kg, 10 mg/kg and 15 mg/kg were found to be safe in all patients. Fifteen adverse events were reported of mild to moderate severity which were considered to be possibly treatment-related. IDEC 131 was not associated with any evidence of toxicity as assessed by the Expanded Disability Status Scale, laboratory parameters, relapse rate and other clinical indications.

Adis Assessment

Study Messages

- IDEC 131 at a dosage of 1 mg/kg, 5 mg/kg, 10 mg/kg or 15 mg/kg is generally well tolerated in patients with relapsing-remitting multiple sclerosis.

Adis Evaluation

Trial Design:

Clinical Relevance:	Provides new evidence of clinical benefit and has major implications for a patient population.

Reference

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IDEC-131. IDEC/Eisai.

Dumont FJ.

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IDEC, in collaboration with Eisai, is developing IDEC-131 (E6040), a humanized monoclonal antibody (mAb) against CD154, the ligand for CD40 also called CD40L or gp39, for the potential treatment of several autoimmun diseases. IDEC-131 is based on technology that IDEC licensed from Dartmouth Medical School where researchers demonstrated the biological effects of the anti-CD154 antibody in animal models of autoimmunity. In January 2001, phase II trials in psoriasis and idiopathic thrombocytopenic purpura (ITP) were initiated. By january 2002, a phase II trial in Crohn's disease was also ongoing. A pilot, multicenter, multiple-dose phase I trial in moderate-to-severe psoriasis was initiated in January 2001. This trial was ongoing in January 2002. IDEC, in collaboration with Dartmouth Medical School had also initiated a phase I trial in multiple sclerosis by March 1999. IDEC-131 was also previously being developed for systemic lupus ezythematosus (SLE), although no further development for this indication ha been reported since the disclosure of disappointing phase II results in April 2000. Analysts at Morgan Stanley predicted in February 2002, that the product would be launched in 2005, with sales of US $25 million, rising to US $75 million in 2006.

Publication Types:

- Review
- Review, Tutorial

PMID: 12090546 [PubMed - indexed for MEDLINE]

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1: Arthritis Rheum. 1991 May;34(5):525-36. Related Articles, Linl

Treatment of rheumatoid arthritis with monoclonal CD4 antibody M-T151. Clinical results and immunopharmacologic effects in an open study, including repeated administration.

Reiter C, Kakavand B, Rieber EP, Schattenkirchner M, Riethmuller G, Kruger K.

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Recent experimental and clinical data point to the T helper lymphocyte subse as playing a central role in the pathogenesis of rheumatoid arthritis (RA). Thus, a therapeutic strategy aimed specifically at the CD4 T cell subset is warranted. We treated patients with active RA for 7 days with a daily dose of 20 mg of CD4 monoclonal antibody M-T151, administered intravenously over 30 minutes. There were no negative side effects. According to changes in the combined parameters of Ritchie articular index, pain assessment, grip strength, and morning stiffness, 6 patients had a good response. Clinical improvement was greatest approximately 2 weeks after termination of the therapy and lasted from 4 weeks to 6 months. Of the serologic parameters of inflammation, only the C-reactive protein level improved in the patients with a favorable response. Close immunologic monitoring revealed a transient, selective depletion of CD4+ T cells after each infusion. During the entire treatment period, residual circulating CD4+ cells were found to be coated with CD4 antibody, whereas free antibody was detected in the serum only fo approximately 8 hours after each infusion. Immediately after infusion, solubl CD4 antigen appeared in the serum. In addition to the cell-bound CD4 antibody, complement components could be detected on the surface of the remaining CD4+ cells. The proliferative response of peripheral blood mononuclear cells to purified protein derivative was significantly diminished 4 weeks after cessation of antibody treatment. Six patients showed a weak antibody response to mouse immunoglobulin. In 4 of the responders who received a second course of therapy (2 of them as outpatients), a therapeutic effect was noted that was similar to that after the first course. Only 1 patient, who had low titers of serum IgE anti-mouse Ig antibodies, showed a mild anaphylactic reaction at the end of the second course of therapy. Treatment o RA with the monoclonal CD4 antibody M-T151 seems to be a promising alternative, although the optimal dose and the regimen of administration are still to be defined.

1: J Neuroimmunol. 1992 Nov;41(1):61-70.

Specific immunotherapeutic strategy for myasthenia gravis: targeted antigen-presenting cells.

Reim J, McIntosh K, Martin S, Drachman DB.

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The pathogenesis of myasthenia gravis (MG) involves a T cell-dependent antibody-mediated autoimmune response directed against acetylcholine receptors (AChR). Inactivation of AChR-specific T cells should interrupt the immune response, resulting in therapeutic benefit. Since each individual's repertoire of T cells responds to a heterogeneous and unique spectrum of AChR epitopes presented in association with self-major histocompatibility complex (MHC) class II, an individualized approach is required to target all relevant AChR-specific T cells. The individual's own antigen-presenting cell (APC) can be used for this purpose, since they process and present the antige appropriately, and express the correct MHC class II. A novel method of binding AChR to surface immunoglobulin with a heterobifunctional antibody conjugate allows us to use all B cells as APC. Conjugate-plus-AChR-treated B cells (AChR-APC) effectively targeted AChR-specific T cells, stimulating vigorous proliferative responses in a rat cell culture system. If APCs are 'fixed' with cross-linking reagents, they induce long-lasting or permanent 'anergy' of the specific T cells. We prepared AChR-APC, allowed them to process AChR in vitro, and fixed them with paraformaldehyde. Pre-culture o these fixed AChR-APC with AChR-specific T cells induced anergy: when restimulated with fresh AChR-APC, the T cells exhibited markedly reduced proliferative responses and IL-2 production, compared with responses of T cells pre-cultured with control fixed B cells. Implications for the design of antigen-specific therapeutic strategies for MG and other immune disorders will be discussed.

PMID: 1460093 [PubMed - indexed for MEDLINE]

1: Med Hypotheses. 1992 Dec;39(4):356-9. Related Articles, Linl

Cytokines play a central role in the pathogenesis of systemic lupus erythematosus.

Singh AK.

Division of Nephrology, Tufts-New England Medical Center, Boston, Massachusetts 02111.

Excessive production of pathogenic autoantibodies is one of the hallmarks of systemic lupus erythematosus (SLE). The mechanisms that underlie this excessive production are still unclear. Although there is considerable evidence to suggest that both T cells and B cells play an important role in the etiology of SLE, convincing abnormalities at the T cell receptor or immunoglobulin gene loci have not been demonstrated. In this regard, because cytokines play such a pivotal role in the inflammatory response, a defect in the immunoregulation of B cells by cytokines should be considered as possible contender in disease etiology. The hypothesis that is proposed here is that multiple defects mediated by cytokines are present in individuals with lupus and that both cytokine production and the response of B cells to cytokines may be defective. These abnormalities could then be a central factor in the etiology of systemic lupus erythematosus.

Publication Types:
- Review
- Review, Tutorial

PMID: 1494323 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1990 Jul;71(1):170-8.

The regulatory role of human helper T-cell clones on antithyroi antibody production by peripheral B-cells.

Graves' Disease

Fisfalen ME, Soltani K, Janiga AM, Kawakami Y, Macchia E, Quintans J, DeGroot LJ.

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The helper effects of thyroid antigen-specific T-cell clones (TCC) on antibody production by peripheral B-cells were studied and compared with similar effects of self major histocompatibility complex II (MHC-II)-reactive TCC as well as uncloned CD4+ cells. Ten TCC were derived from thyroid tissue or peripheral blood mononuclear cells (PBMC) in patients with Graves disease. Uncloned CD4+ cells were also obtained from PBMC in patients with autoimmune thyroid disease. All TCC were CD3+/CD4+. B-Cells from patients with mainly high serum levels of microsomal antibodies (McAb) were cultured alone and with either TCC or uncloned CD4+ cells in the presence or absence of thyroid antigens [microsomal antigen/thyroid peroxidase (McAg/TPO) and thyroglobulin (Tg)] or pokeweed mitogen (PWM). Total immunoglobulin G (IgG) and specific thyroid antibodies were measured by enzyme-linked immunosorbent assay. Self MHC-II-reactive TCC induced B-cell production of total IgG and even McAb independent of antigens or PWM. Specific TCC required thyroid antigens to induce antibodies. The optimal McAg/TPO or Tg concentration was 10 ng/mL for total IgG production and 1 ng/mL McAg/TPO for McAb synthesis. The addition of PWM did not affect McAb production, but enhanced total IgG synthesis by B-cells under the influence of some specific TCC. Uncloned CD4+ cells induced both total IgG and McAb synthesis in the presence of PWM. With thyroid antigens, uncloned CD4+ cells induced total IgG synthesis at levels comparable to those of specific TCC, but induced smaller quantities of McAb in the presence of McAg/TPO. Our antigen-specific TCC could, therefore, stimulate specific B-cells to produce thyroid antibodies in vitro. Self MHC-II-reactive TCC could also induce specific antibodies by B-cells. Both self MHC-II-reactive CD4+ cells and antigen-specific CD4 cells may play an important role in the pathogenesis and/or perpetuation of autoimmune thyroid disease.

PMID: 1695223 [PubMed - indexed for MEDLINE]

1: Autoimmunity. 1992;13(4):311-9.

Cellular immune mechanisms in chronic autoimmune thrombocytopenic purpura (ATP).

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Chronic autoimmune thrombocytopenic purpura (ATP) is a common autoimmune-mediated bleeding disease in which autoantibodies are directed against platelets, resulting in their enhanced Fc-mediated destruction by macrophages in the spleen. While there has been extensive studies relating tc the autoantibodies in this autoimmune disorder, relatively few have dealt wit cell-mediated immunoregulation of the anti-platelet autoantibody response. Nonetheless, there is accumulating evidence that suggests the production of these anti-platelet autoantibodies is under the influence of several abnormal lymphocyte-mediated mechanisms, i.e. enhanced anti-platelet T helper cell activity with concomitant reduced T suppressor cell activity. This review focuses on these cellular events and presents a working model which attempt to explain their close interrelationships.

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Predictive value of islet cell and insulin autoantibodies for type 1 (insulin-dependent) diabetes mellitus in a population-based study of newly-diagnosed diabetic and matched control children

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Most studies evaluating immune markers for prediction of Type 1 (insulin-dependent) diabetes mellitus have focused on first degree relatives, although only 10% of newly-diagnosed patients have an affected first degree relative. The Swedish Childhood Diabetes Register identifies 99% of all diabetic children at diagnosis. In this population-based study, islet cell antibodies and insulin autoantibodies in 0-14-year-old Swedish consecutively-diagnosed patients and control subjects were analysed to define their sensitivity and specificity. Over 16 months (1986-1987), 515 Swedish children developed diabetes. Plasma samples were obtained from 494 (96%) patients, and 420 matched control children. Among patients, the frequency of islet cell antibodies was 84% (415 of 494), insulin autoantibodies 43% (145 of 334); 40% (135 of 334) were positive for both and 88% (294 of 334) were positive for one or both. Among control children, 3% (14 of 420) had islet cell antibodies, 1% (4 of 390) insulin autoantibodies, and 4% (16 of 390) had either autoantibody marker. The predictive value of finding a patient with the disease was only 7% since 4% of the control children were antibody-positive and the cumulative incidence rate up to 15 years of age is 0.38%. None of the autoantibody-positive (n = 21) or negative control children developed diabetes during 3 to 5 years of follow-up. Longitudinal investigations of islet cell or insulin-autoantibody-positive healthy children are necessary to accurately determine the conversion rate from marker positivity to disease onset.

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Prediction and prevention of type 1 diabetes

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Clinical type 1 diabetes represents end-stage insulitis resulting from progressive β-cell destruction over an asymptomatic period that may last for years. This knowledge and recent advances in our ability to identify individuals at increased risk for clinical disease have paved the way for trials aimed at preventing or delaying the clinical onset of type 1 diabetes. Individuals at risk for type 1 diabetes can be identified by a positive family history, or by genetic, immunological or metabolic markers. These markers can also be combined to achieve a higher positive predictive value. As long as there is no effective preventive modality available for clinical use, screening for the identification of risk individuals can be considered ethically acceptable only in the context of sound research protocols. Prevention of type 1 diabetes can be implemented at three different levels, out of which primary prevention includes all strategies aimed at decreasing the risk of developing type 1 diabetes in individuals without any signs of β-cell damage. Secondary prevention aims to reduce the incidence of type 1 diabetes by stopping β-cell destruction in individuals with signs of such a process, while the objective of tertiary prevention is to restore β-cell function or prevent complications in patients with overt type 1 diabetes. At present, one primary prevention trial and four comprehensive secondary prevention trials are in progress. Common features of these intervention trials are that the recruitment of patients fulfilling the inclusion criteria is time-consuming and the trials must proceed for a long time, as clinical disease is the end point. The secondary prevention trials also require extensive screening for the identification of eligible patients. The ongoing intervention trials may, however, represent a new era in type 1 diabetes, i.e. the beginning of the end of this complicated disease. □ *Autoantibodies, genetic markers, prediction, prevention, type 1 diabetes*

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Type 1 diabetes is perceived as a T-cell-mediated autoimmune disease characterized by progressive β-cell destruction during an asymptomatic preclinical period (1–3). Clinical type 1 diabetes represents end-stage insulitis, as it has been estimated that 80–90% of the insulin-producing β-cells have already been damaged at the time of diagnosis. The insight that the clinical manifestation of type 1 diabetes is preceded by an asymptomatic phase that may last for years, and recent advances in our ability to identify individuals at increased risk of progressing to clinical disease, have opened doors for trials aimed at preventing or delaying the clinical onset of type 1 diabetes.

If no safe intervention measures are available that can be implemented in the general population, successful prevention of type 1 diabetes has at least two preconditions. First, one must be able to identify individuals at increased risk for progression to type 1 diabetes and, secondly, one must have an intervention modality with less severe adverse effects than those associated with the disease itself. This paper reviews the present status in the dynamic area of the prediction and prevention of type 1 diabetes.

Prediction of type 1 diabetes

There are different approaches for the identification of individuals at risk for type 1 diabetes. These approaches are based on the family history of type 1 diabetes, genetic disease markers, autoimmune markers or metabolic markers of type 1 diabetes. These alternatives may also be combined in various ways to improve the predictive characteristics of the screening strategy.

Family history

It has been shown that offspring of mothers with type 1 diabetes have a risk of about 2–3% of developing clinical type 1 diabetes before adulthood, whereas the risk is approximately 5–6% in offspring of affected fathers (4–6). So far, no satisfactory explanation has been presented for the higher risk associated with paternal disease. In brothers and sisters of children with type 1 diabetes, the risk of developing the disease is clearly related to their degree of HLA identity with the index case. The average risk has been estimated to be 6–8%, increasing to 16–20% in HLA-identical sibs and decreasing to close to 1% in non-HLA-identical sibs (7–9). Altogether, only about 10% of children with type 1 diabetes have an affected family member at the time of diagnosis (5, 7, 10). Accordingly, this approach has a low sensitivity for type 1 diabetes at the general population level and does not provide a clinically useful tool alone,

although it is without doubt the cheapest of the available methods.

Genetic markers

Recent human genome-wide searches for genes associated with type 1 diabetes susceptibility have clearly shown that this is a multifactorial disease with a polygenic predisposition (11, 12), although the HLA gene region on the short arm of chromosome 6 comprises the major locus conferring disease susceptibility. In addition to this locus, the genome-wide search identified more than 15 other chromosomal regions that may contribute to the genetic predisposition to type 1 diabetes. It has been estimated that the HLA genes explain 40–60% of the genetic susceptibility to type 1 diabetes in various populations, and the contribution of the non-HLA genes also varies from 40 to 60% (Todd JA, pers. comm.). The HLA class II antigens, DR3 and DR4, are the classical genetic markers of type 1 diabetes, the latter being dominant in northern Europe and the former in southern Europe. More recently, the HLA molecules, DQ2 and DQ8, have been shown to have a somewhat stronger disease association than the corresponding DR antigens, although the DQ-defined predisposition is modified by various DR4 subtypes (13).

The relative and absolute risks associated with various HLA DQB1 genotypes have been studied in Finland (14). The highest risk was seen in those carrying the HLA DQB1*02/0302 genotype with a relative risk of 11 and an absolute type 1 diabetes risk of about 10% before the age of 20 y. The DQB1*0302/x genotype (where x stands for *0302 or a neutral allele) was associated with a relative risk of 5 and an absolute risk of 3–4% before adulthood. By analysing four DQB1 alleles, a proportion of the general population carrying an absolute risk of 5% for type 1 diabetes before the age of 20 y could be identified. Their risk is, therefore, close to that seen in sibs of children with type 1 diabetes. This high-risk group comprises approximately 14% of the general population and covers about 70% of future type 1 diabetes patients in Finland. This strategy for genetic screening is used in the ongoing Diabetes Prediction and Prevention (DIPP) study (15). A similar approach can also be used in other countries with a Caucasian population, although the proportion of future patients with type 1 diabetes and the absolute disease risk may vary from one country to another.

Autoimmune markers

Humoral autoimmunity against pancreatic islets was first described in 1974 by Bottazzo et al., when they reported that antibodies to the pancreatic islets could be detected in sera of patients with type 1 diabetes and polyendocrine diseases (16). Later, it was shown that most patients with type 1 diabetes have these islet cell antibodies (ICA) in their sera at diagnosis and during the preclinical phase of the disease (17–20). The association of insulin autoantibodies (IAA) with type 1 diabetes was reported in 1983, when Palmer et al. described the presence of IAA in 18% of untreated patients with newly diagnosed type 1 diabetes (21). Subsequently, IAA have been observed to be inversely related to age in patients with newly diagnosed type 1 diabetes (22, 23), with a frequency of up to 80% in those diagnosed before the age of 5 y and a prevalence of less than 30% in patients with clinical disease manifestation at adult age. Antibodies to a 64 000 mol. wt islet cell protein were described in type 1 diabetes sera in 1982 (24). This protein was later shown to be identical to the enzyme glutamic acid decarboxylase (GAD) and to be one of the major antigens for autoantibodies in sera from patients with preclinical or clinical type 1 diabetes (25–28). Antibodies to the intracellular fragment of a molecule belonging to the family of protein tyrosine phosphatases (IA-2, ICA512) have recently been shown to be associated with type 1 diabetes (29–31).

Several studies have evaluated the predictive characteristics of single and combined autoantibody specificities in first-degree relatives of patients with type 1 diabetes (32–35). The results are concordant in that the risk for type 1 diabetes increases as a function of the number of autoantibodies detectable and of the levels of autoantibodies, with the exception of GAD antibodies (36). It is possible to achieve a positive predictive value of 60–80% and a disease sensitivity of 60–80% over a follow-up period of 5 y

Table 1. Predictive characteristics of single autoantibody specificities and antibody combinations in 755 initially unaffected sibs of children with type 1 diabetes observed up to the diagnosis of type 1 diabetes or for a minimum of 7 y in the Childhood Diabetes in Finland (DiMe) study (36).

Marker	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
ICA	81	95	43	96
IAA	25	97	29	97
GAD antibodies	69	96	42	99
IA-2 antibodies	69	98	55	99
GAD and/or IA-2 antibodies	81	98	41	99
One antibody	3	94	2	96
Two antibodies	9	99	25	96
Three or four antibodies	72	98	66	99

ICA: Islet cell antibodies; IAA: insulin autoantibodies; GAD: glutamic acid decarboxylase; IA-2: a protein tyrosine phosphatase-related protein.

based on these studies. Table 1 presents the predictive characteristics of various autoantibodies and antibody combinations observed in the Childhood Diabetes in Finland (DiMe) study (36). This is a population-based study of children diagnosed with type 1 diabetes in 1986–89 and their families. One arm of the study comprises observation of 755 initially unaffected sibs from the time of diagnosis in the index case, up to the clinical manifestation of type 1 diabetes or for a minimum of 7 y. During this time, 32 sibs (4.2%) progressed to clinical type 1 diabetes. The classical ICA turned out to be the most sensitive single antibody test, while IAA gave the lowest sensitivity. The combination of GAD and IA-2 antibodies resulted in a sensitivity identical to that of ICA. IA-2 antibodies had the highest specificity and also the highest positive predictive value out of the single antibody tests. Positivity for at least three antibodies out of four was associated with the highest positive predictive value for type 1 diabetes and satisfactory sensitivity and specificity.

Whether it is possible to transfer these predictive characteristics directly from first-degree relatives to the general population remains unclear. Some data have indicated that ICA have a substantially lower predictive value in unaffected children from the background population than the predictive value in sibs of young patients with type 1 diabetes (37). However, data from the background childhood population indicate that positivity for multiple type 1 diabetes-associated autoantibodies is quite a rare phenomenon, suggesting that a predictive strategy based on multiple autoantibodies may potentially carry a high positive predictive value in the general population (38, 39). It should not be forgotten, however, that the emergence of autoantibodies is a dynamic process (3), and so far there is no consensus on at what age and how often children in the general population should be tested to achieve optimum sensitivity.

Antigen-specific T-cell responses to β-cell antigens have also been described in both patients with newly diagnosed type 1 diabetes and prediabetic subjects (40–42). At the moment, however, not enough data are available to judge whether markers of cell-mediated autoimmunity have a potential for facilitating the identification of individuals at high risk of the disease. The results of the first International Workshop on Standardization of T-cell Responses were disappointing from this point of view that no consistent differences were reported in antigen-specific T-cell responses between patients with type 1 diabetes and controls (Roep BO, pers. comm.). In addition, the more complicated sampling and test protocols for analysing phenomena reflecting cell-mediated autoimmunity, compared with autoantibody tests, make these parameters impractical in studies with high numbers of individuals at risk.

Metabolic markers

A decreased first-phase insulin response (FPIR) to intravenous glucose has been shown to be the most sensitive marker of a reduced insulin secretory capacity when studying autoantibody-positive, first-degree relatives of patients with type 1 diabetes (43, 44). A lost FPIR may represent the point of no return in subjects with preclinical disease. More recent data suggest, however, that non-progressive, subclinical β-cell dysfunction may be relatively common in family members of affected patients (20, 45). No extensive study aimed at determining the FPIR in normal children of various ages has been performed, and therefore the reference values used are not necessarily adequate. Although a short (10 min) intravenous glucose tolerance test (IVGTT) is sufficient for the assessment of the FPIR, this is still a rather invasive and labour-intensive procedure, which limits its applicability in the estimation of risk in young children in particular.

In a study of 23 antibody-positive sibs of children with type 1 diabetes, it was observed that the 11 with a reduced FPIR were characterized by decreased fasting proinsulin:insulin and proinsulin:C-peptide ratios (46), and 9 of these children progressed to clinical disease within 28 months. There was also an inverse correlation between the FPIR and the fasting proinsulin:C-peptide ratio in the whole study group, suggesting that a high fasting proinsulin:C-peptide ratio may be an indicator of reduced insulin secretory capacity.

Practical implications

An optimal screening test for type 1 diabetes should have high sensitivity and specificity, combined with a high positive predictive value. It should also be reproducible, inexpensive and not too invasive. Of the screening tools discussed above, none is able to fulfil all of these criteria, so compromises have to be sought. Families with a recent-onset diabetic child tend to want to know the risk for the remaining sibs. If risk assessment is indicated, the most feasible strategy is to screen the sibs for type 1 diabetes-associated autoantibodies, as it is possible to make a relatively reliable assessment based on the number of antibodies present. This assessment may be further strengthened by performing a short IVGTT in those sibs who test positive for at least one disease-associated antibody.

Ethical and psychological aspects

Is screening for type 1 diabetes in families with at least one affected member or in the general population ethically justified? So far, the consensus has been that screening is ethically acceptable only within the framework of a well-designed research protocol. As reliable identification of high-risk individuals has come substantially closer, however, there may be a need to reconsider this issue. What, then, are the advantages of extensive screening? One definite benefit is that in most cases, in more than 85% of siblings and more than 95% of children from the background population, families can be informed that their child has a low or decreased risk of type 1 diabetes. Another advantage is that a child identified as being at high risk may

Table 2. Prevention trials in progress

Trial	Intervention modality	Target group	Inclusion criteria	Estimated risk of type 1 diabetes	Study design	Results available
TRIGR	Primary intervention: elimination of cow's milk proteins up to at least the age of 6 months	Newborn infants from diabetic families (n = 2800)	HLA DQB1*0301 and/or *0201, but not *0602, *0603 or *0301	12% within 10 y	Randomized, controlled, double-blind	Second pilot study 1999; Study proper 2010?
New Zealand Nicotinamide Study	Secondary prevention: nicotinamide	Schoolchildren, 5–7 y old at initial screening (n = 173)	ICA ≥ 20 JDF units or ≥ 10 JDF units and FPIR < 25th percentile	?	Open, controlled	Indefinite follow-up?
ENDIT	Secondary prevention: nicotinamide	First-degree relatives (n = 528)	ICA positivity in two sequential samples, one with a level ≥20 JDF units Age 5–40 y	35% within 5 y	Randomized, controlled, double-blind	2003
DPT-1	Secondary prevention: (A) Subcutaneous + oral insulin	First- and second-degree relatives (n = 255)	ICA ≥10 JDF units Age < 45 y FPIR < 10th percentile Excluded: HLA DQB1*0602	>50% within 4 y	Open, controlled	After 2002
	(B) Oral insulin	First- and second-degree relatives (n = 159)	ICA ≥10 JDF units Age <45 y FPIR >10th percentile Excluded: HLA DQB1*0602	<50% within 4 y	Randomized, controlled, double-blind	After 2002
DIPP	Secondary prevention	Children from the general population with increased genetic risk (HLA DQB1*02/0302 or *0302/x) (n = 170)	Age >1 y Positive for at least two type 1 diabetes-associated antibodies in two consecutive samples	Approx. 50% within 5 y	Randomized, controlled, double-blind	After 2005

ICA: Islet cell antibodies; FPIR: first-phase insulin response; JDF: Juvenile Diabetes Foundation.

- adenine dinucleotide (NAD), which prevents cellular destruction;
- it inhibits cytokine-induced nitric oxide production in the islets and prevents cytokine-induced major histocompatibility complex (MHC) class II expression on cultured islet cells.

The relevance of the population-based nicotinamide intervention trial in New Zealand has been widely discussed because of a somewhat unorthodox study design and lack of randomization. The design has, however, been confirmed to be valid by statistical expertise. Recent results from the trial suggest a prolonged and considerable (56%) protective effect of nicotinamide after an average follow-up of 7.1 y (52).

The European Nicotinamide Diabetes Intervention Trial (ENDIT) is an international multicentre study with a randomized, controlled design. The trial is testing whether it is possible to decrease the incidence of type 1 diabetes by 40% (i.e. from 35% down to 20%), over 5 y in high-risk, first-degree relatives using daily oral administration of slow-release nicotinamide at a dose of 1200 mg $m^{-2} d^{-1}$. The study population needed for the trial has been estimated to be 528 family members, with a statistical power of 90% and an expected drop-out rate of 20%. The target population was reached in December 1997. The first and only interim analysis will be performed in autumn 1998, when half of the participants have passed the first 2.5 y of the study. The final outcome will be analysed at the beginning of the year 2003.

The German nicotinamide intervention trial, Die Deutsche Nikotinamid-Interventions-Studie (DENIS), was terminated in March 1997, as it was unlikely to reach the statistically significant result specified in the hypothesis (57). The study was designed, however, to detect an 80% reduction in the incidence of type 1 diabetes (a reduction from 30% to 6%) with a power of 90%. The negative result of DENIS does not, however, exclude a treatment effect of less than 80%.

One common critical issue for all nicotinamide studies is the relationship between the achievable circulating peak concentration of the compound and biologically effective nicotinamide concentrations. A serum peak concentration of about 0.1 mmol l^{-1} can be obtained with a dose of 25–30 mg kg^{-1}, which is equivalent to the doses used in the two ongoing human studies. This concentration is sufficient for a 50% inhibition of the PARP activity, but a concentration about 10 times higher (1 mmol l^{-1}) is needed to be able to restore NAD and the synthesis of proinsulin in streptozotocin-treated islets. To counteract cytokine-induced effects on nitric oxide production and MHC class II expression *in vitro*, concentrations of 10–100 mmol l^{-1} are required. There was a suggestion in the past, however, that nicotinamide accumulated in the pancreatic islets (58), although this observation has not been confirmed using modern techniques.

Another issue of concern is whether nicotinamide can induce insulin resistance. A recent study from the USA reported that short-term nicotinamide administration resulted in a 24% decrease in insulin sensitivity in ICA-positive, first-degree relatives of patients with type 1 diabetes (59). A meta-analysis of 10 randomized nicotinamide intervention trials in patients with newly diagnosed type 1 diabetes showed higher serum C-peptide concentrations after a disease duration of 1 y in those treated with nicotinamide than in controls, whereas no significant differences could be seen in the metabolic control or exogenous insulin dose between these two groups (60). This observation could be explained by decreased insulin sensitivity in the patients given nicotinamide. There are indications that nicotinic acid from which nicotinamide is derived may induce insulin resistance and glucose intolerance (61). It is also known that most commercially available nicotinamide preparations contain small amounts of nicotinic acid. Thus, the observed harmful effect of nicotinamide on insulin sensitivity may be due to contamination with trace amounts of nicotinic acid.

The American Diabetes Prevention Trial 1 (DPT-1) comprises two arms (Table 2). In the first arm, relatives with a risk for type 1 diabetes in excess of 50% over the next 4 y are treated with parenteral insulin, while relatives with a type 1 diabetes risk of less than 50% are treated with oral insulin. The parenteral insulin treatment comprises two daily injections of intermediate-acting insulin (0.25 IU $kg^{-1} d^{-1}$) and a 4-d i.v. infusion of insulin given annually. The dose of insulin in the oral arm is 7.5 mg d^{-1} (approximately 180 IU d^{-1}). The first arm of the DPT-1 study is being implemented as an open, randomized study where the controls are simply observed with no intervention. The study was initiated in 1995 with a target of 255 patients. By the end of October 1977, 200 participants had been randomized (Skyler J, pers. comm.). The most serious concerns in this trial are related to the risk of severe hypoglycaemia in the treated group. Clinicians taking care of patients with recent-onset type 1 diabetes know that some patients encounter hypoglycaemia when treated with insulin doses similar to those used in DPT-1, and a few of these hypoglycaemic episodes are severe. The second arm of DPT-1 started in 1996 and the recruitment target is 159 relatives. A total of 106 patients had entered the study by the end of October 1997. This trial addresses an area with a series of unanswered questions. No scientific data are available on what would be an optimal dose of oral insulin to prevent human type 1 diabetes, and how often and for how long insulin should be given. The most serious objections to oral treatment with insulin arose recently, when an Australian report implied that oral administration of large amounts of ovalbumin to transgenic mice expressing ovalbumin in their β-cells led to the generation of $CD8^+$ cytotoxic T-cells capable of inducing autoimmune diabetes (62). However, it has been shown that feeding insulin to young NOD mice delays the onset and reduces the incidence of autoimmune diabetes in these animals (63).

The Finnish DIPP study is targeting the general population. All infants born in three university hospitals in Finland

are screened for HLA DQB1 markers from cord blood samples and those families with an infant with increased genetic risk (i.e. carrying the HLA DQB1*02/0302 or *0302/x genotype, where x stands for alleles other than *02, *0602 or *0301) are invited to take part in an observational study with sequential sampling for the analysis of type 1 diabetes-associated autoantibodies at an interval of 3–12 months, up to the age of 10 y. Those children who present with at least two autoantibodies in two consecutive samples will then be invited to join an intervention trial based on daily administration of intranasal insulin at a dose of 1 IU kg^{-1}. To test the hypothesis of whether it is possible to delay the clinical manifestation of type 1 diabetes by 3 y at a statistical power of 80% and a drop-out rate of 20%, the trial needs a study population of 170 children. The intervention arm started in autumn 1997 and 11 patients had been randomized by the end of 1997. The recruitment target is expected to be reached in 2002, implying that the outcome of the trial will be available by 2005. Many of the concerns associated with the use of oral insulin are also valid for intranasal insulin. A pilot study has been performed for 6 weeks in healthy adults. There were no signs of hypoglycaemia induced by intranasal insulin and no histological or functional changes were observed in the nasal mucosa over the treatment period.

Conclusions

Substantial progress has been made in the field of prediction and prevention of type 1 diabetes over the last 2 decades. At the beginning of the 1980s it was realized that clinical disease manifestation is preceded by an asymptomatic preclinical period that may last for years, and that ICA are good markers of increased risk for type 1 diabetes in first-degree relatives of affected patients. At that point, no preventive methods were in the pipeline. The number of type 1 diabetes-associated autoantigens and autoantibodies increased towards the end of the 1980s, and it was subsequently shown that the presence of multiple autoantibodies resulted in higher predictive values than ICA positivity alone. The first intervention trials were initiated at the beginning of the 1990s. The next decade can be expected to offer improved assessment of type 1 diabetes risk, in the general population in particular, and assessment of type 1 diabetes risk may be integrated into clinical practice within the next 10 y. At the same time, intervention modalities suitable for clinical use might become available. However, a total eradication of clinical type 1 diabetes cannot be expected in the next century, as it is probable that a combination of different interventions will be needed to achieve an optimum effect. The prevention trials in progress represent a new era of type 1 diabetes, i.e. the beginning of the end of this complicated disease.

When caring for prediabetic children and adolescents, the ancient advice of Hippocrates, "...never do harm to anyone", has to be kept in mind. This emphasizes the fact that planned interventions in children need to have a strong scientific rationale confirmed in experimental models of autoimmune diabetes. In addition, toxicological data from experimental studies as well as from studies in adults should be available to indicate that the modality is safe. Any trial should also be planned and implemented with such a design that it produces scientifically reliable information. This aspect has been stressed by guidelines from both the American Diabetes Association (64) and the International Diabetes Immunotherapy Group. Accordingly, future intervention trials call for broad national and/or international collaboration to facilitate the recruitment of study populations large enough to provide reliable new knowledge. One strategy to shorten the duration of clinical trials for the prevention of type 1 diabetes would be to establish reliable surrogate markers of clinical type 1 diabetes. Such surrogate markers would facilitate the initial evaluation of potential intervention modalities and the selection of the most promising therapies for full-scale testing, with overt disease as the end-point. The route towards effective prevention of type 1 diabetes will hardly be a well-paved highway, but rather a path lined by both success and disappointment. The process has been initiated, however, and we can only hope that it will result in the foreseeable future in a significant decrease in the number of children progressing to clinical type 1 diabetes.

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